RÉPUBLIQUE FRANÇAISE
Liberté Égalité Fraternité

FRANCE 2030 — PROGRAMME DE RECHERCHE
CARBONE ET ÉCOSYSTÈMES CONTINENTAUX

Aix*Marseille université · ENS | PSL · IRD · UNIVERSITÉ DE ROUEN NORMANDIE · CERAAS Centre d'étude régional pour l'amélioration de l'adaptation à la sécheresse · cirad · cnrs · ISRA · cea · INRAE

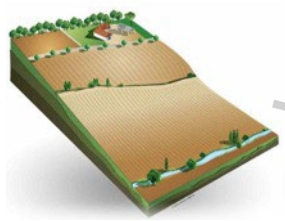
RhizoSeq-C

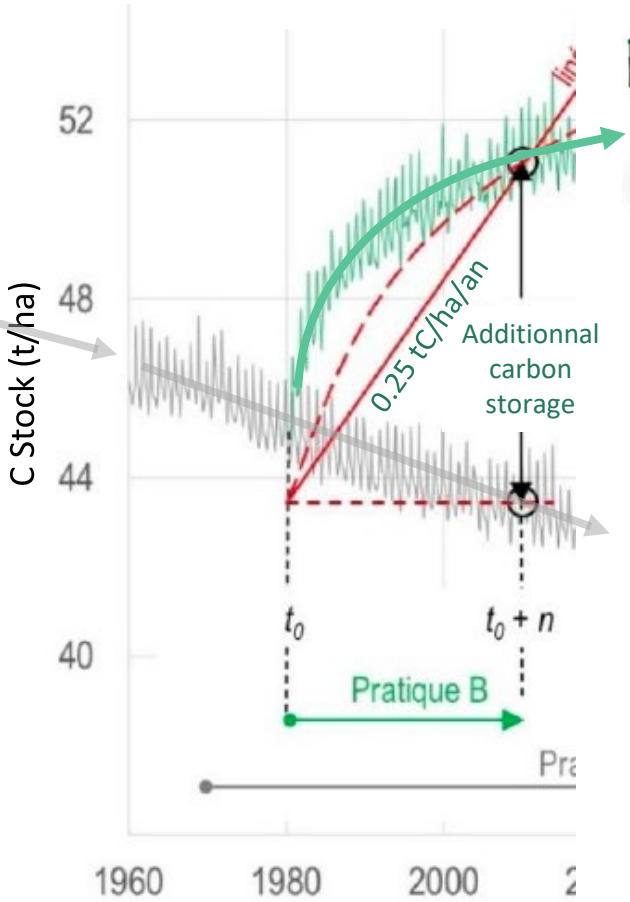
Optimizing rhizodeposition to increase carbon sequestration in agrosystems

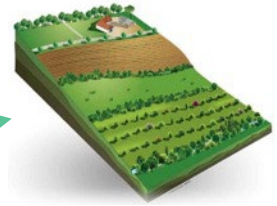
cnrs INRAE

Land management and C stocks

Practice change on crop system

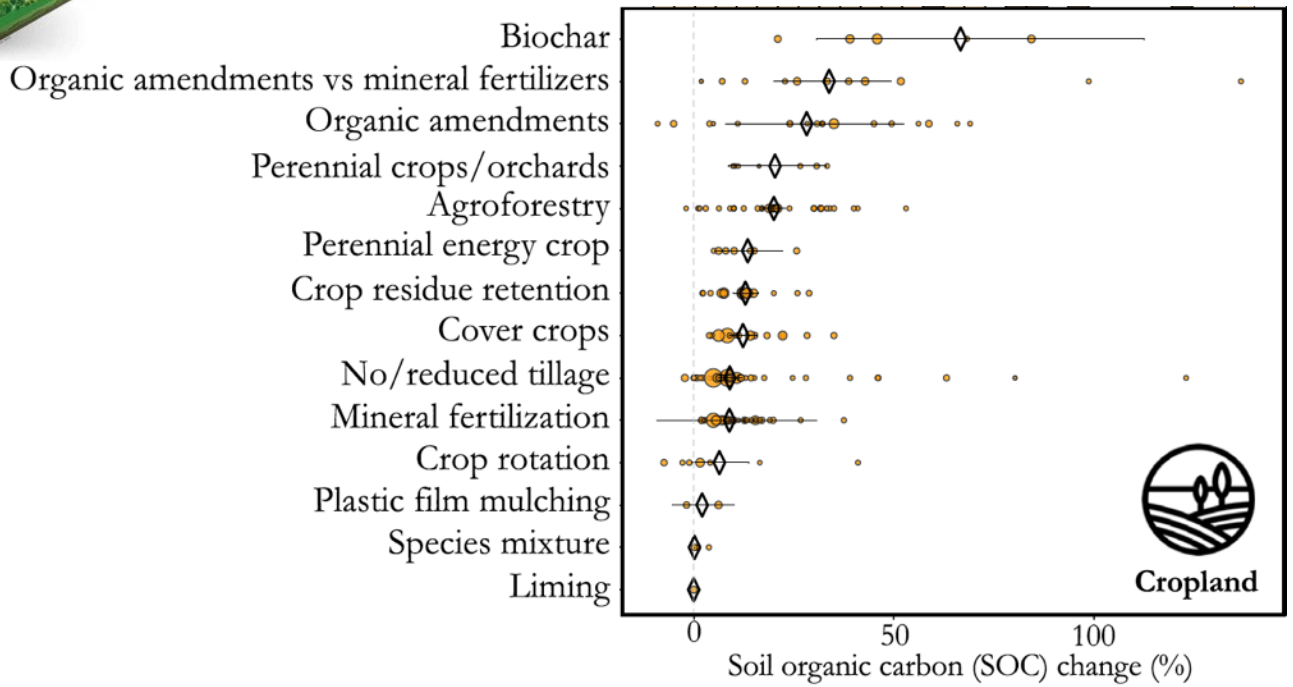






C sequestration in crop soils

↗ 0.28 to 0.43 t C ha^{-1} yr^{-1} *Lessmann et al., 2021*



Belloin et al. 2023

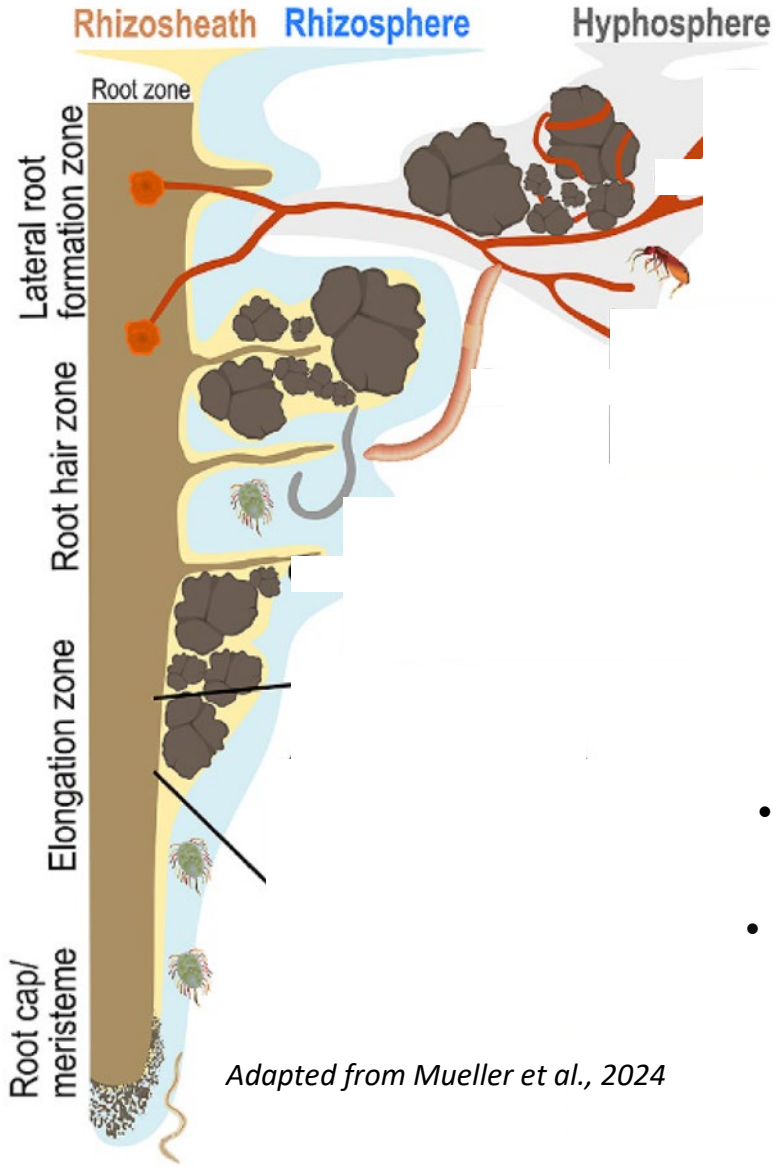
Rational of RhizoSeqC

Plant rhizodeposition is a key factor for soil organic matter formation in stable fractions

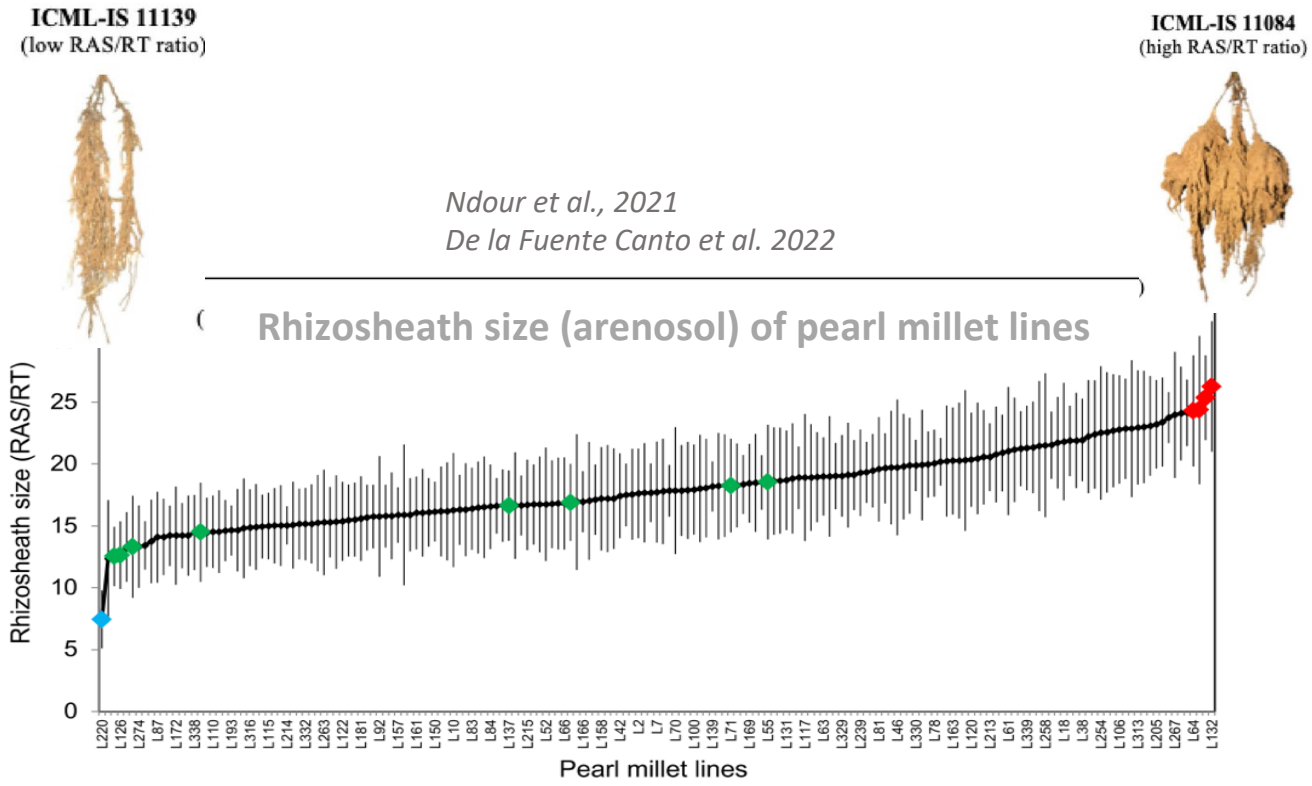
46% of C-inputs incorporated in the mineral-associated organic C fraction

Villarino et al. 2021

Rhizosheath : a proxy of rhizodeposition



Adapted from Mueller et al., 2024



Ndour et al., 2021
De la Fuente Canto et al. 2022

- The size of the rhizosheath varies by a factor of 3 depending on the pearl millet lines
- The size of the rhizosheath is genetically regulated by multiple genes, each exerting a minor effect, contributing to this phenotype

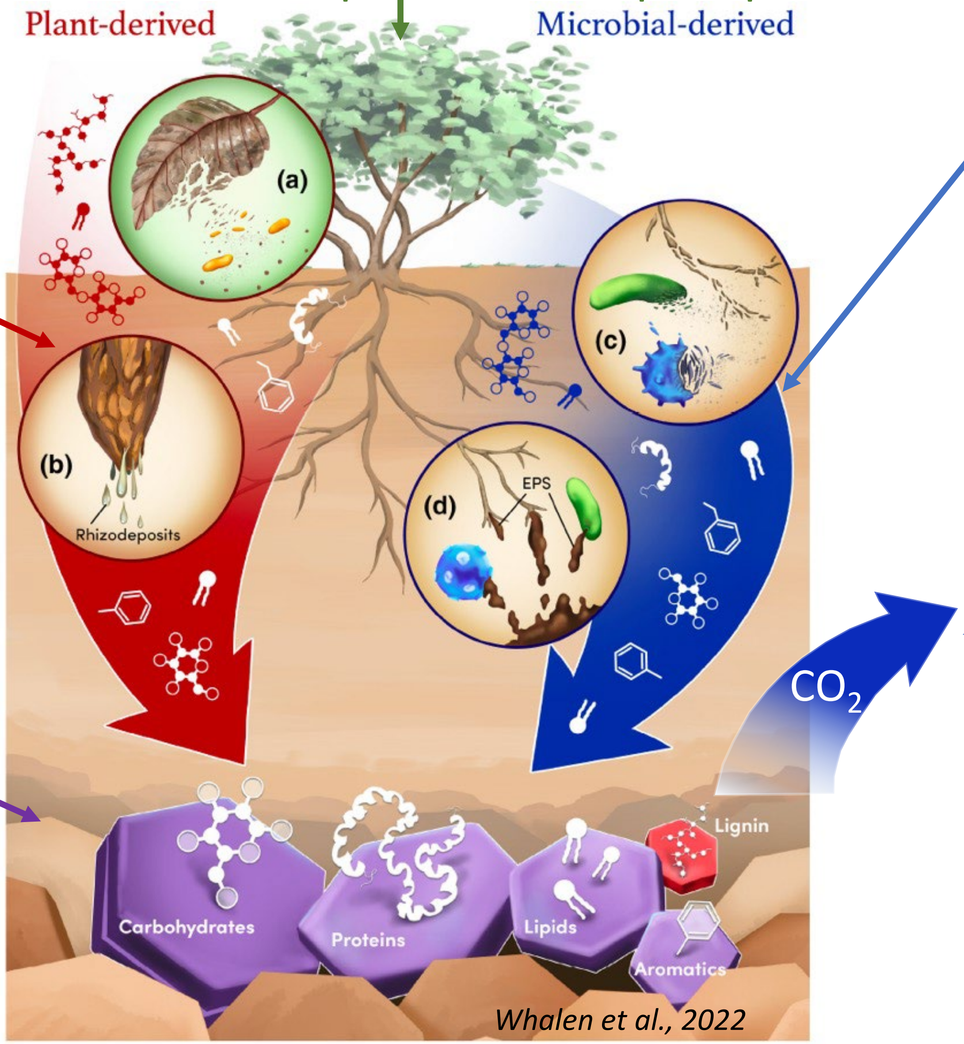
Controls of soil organic matter dynamics

Climate : temperature and precipitation *Steiner et al. 2024*

Rhizodeposits :
major plant C inputs

Villarino et al. 2021

Microbiome : Genotype-dependant (structure and functions) *Alahmad et al., 2024*



Whalen et al., 2022

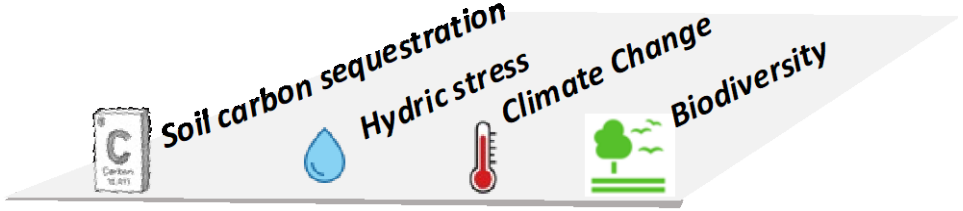
Soil type and **mineralogy :**
Stabilization of organic matter hundreds to thousands of years

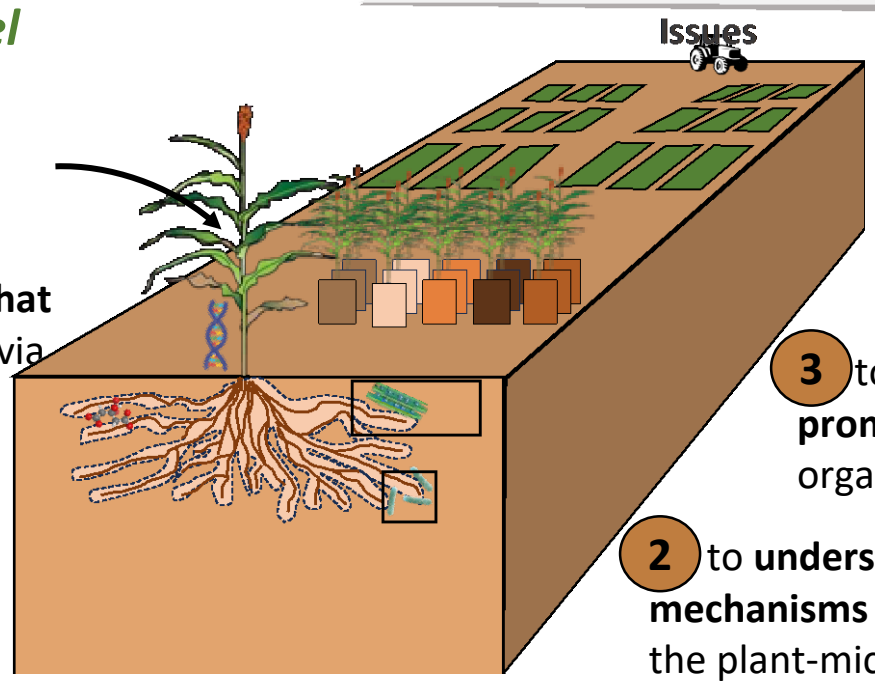
Priming Effect: mean increase of 30% in CO_2 output

Batsida et al., 2019
Bernard et al., 2022

Objectives of RhizoSeqC

General objective : to **develop cropping systems** capable of combining an **increase of direct soil C inputs** and a **promotion of soil OM stabilization** while maintaining **economically attractive production** in contrasted climates





Plant level

1 to identify **the genotypes and genomic regions that promote C inputs** via rhizodeposition

2 to **understand the mechanisms** of C transfer within the plant-microbiome-soil continuum

Agrosystem level

3 to **identify minerals that promote OM stabilization** by organomineral interactions.

4 to **estimate the feasibility** of implementing such cropping systems in contrasted climates

Impact level

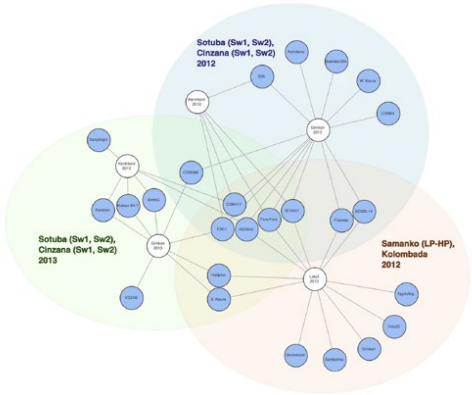
5 to quantify additional soil carbon storage to **assess the sequestration trends in the long term**

6 to structure, on a national and international scale, a **community of multidisciplinary scientists**

Experimental choices : sorghum

- **5th cereal** at the worldwide level
- **High biomass production** for livestock fodder / also used for food (staple food for 300 M°)
- **Mainly produced in Africa** (27 Mha), but sorghum value chains are also **developed in Europe**
- **High water deficit tolerance** : plant of major agricultural interest in a context of climate change
- **Relevant « Elite » genetic materials** : backcross-nested association mapping (BCNAM) populations *(Garin et al., 2024), public-private collaborations*
- **A first study** showed plasticity of root hair and rhizosheath traits (only for for 2 genotypes) *(Adu et al. 2023)*
- **Large variability of rhizosheath traits expected** according to results achieved in pearl millet *(Ndour et al., 2017, 2020, 2021, 2022)*

Sorghum BCNAM trial established in 2014

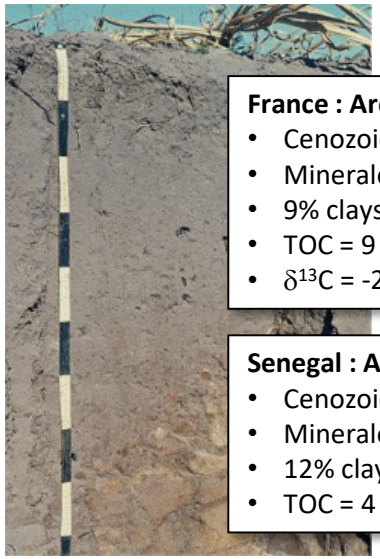


BCNAM Populations and trials in Garin et al., 2024
2,000 individuals developed in the framework of the Biomass For the Future (BFF, PIA4) program

Experimental choices : soils

Arenosols :

- **4th major soil type** (8.5% of land area), notably African areas under high food pressure *(FAO-GIS, 1998)*
- Surprisingly, **poorly studied** *(Kögel-Knabner & Amelung, 2021)*
- Low C content / C stocks highly impacted by land-use change / correlated with **high C deficit** *(FAO-GIS, 1998) (Quero et al. 2022)*
- Arenosols (not aggregative and C poor) are relevant precisely because they are **favourable conditions for revealing (1) the differential aggregative** behaviour of sorghum lines and **(2) the capacity for C stabilisation** by added mineral phases.
- **Expertise** of the teams on arenosols (Senegal, France)
- The results obtained on pearl **millet rhizosheath were obtained on arenosols** *(Ndour et al., 2017, 2020, 2021, 2022)*

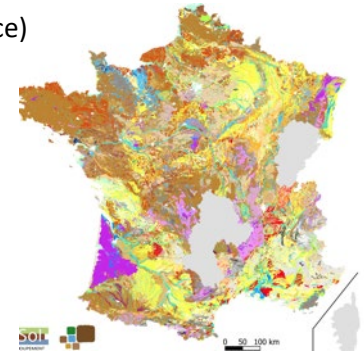


France : Arenosol Nemours
- Cenozoic sandstone parent rock
- Mineralogy dominated by quartz
- 9% clays / 20% silts / 70% sands
- TOC = 9 gC/kg
- $\delta^{13}C$ = -27‰

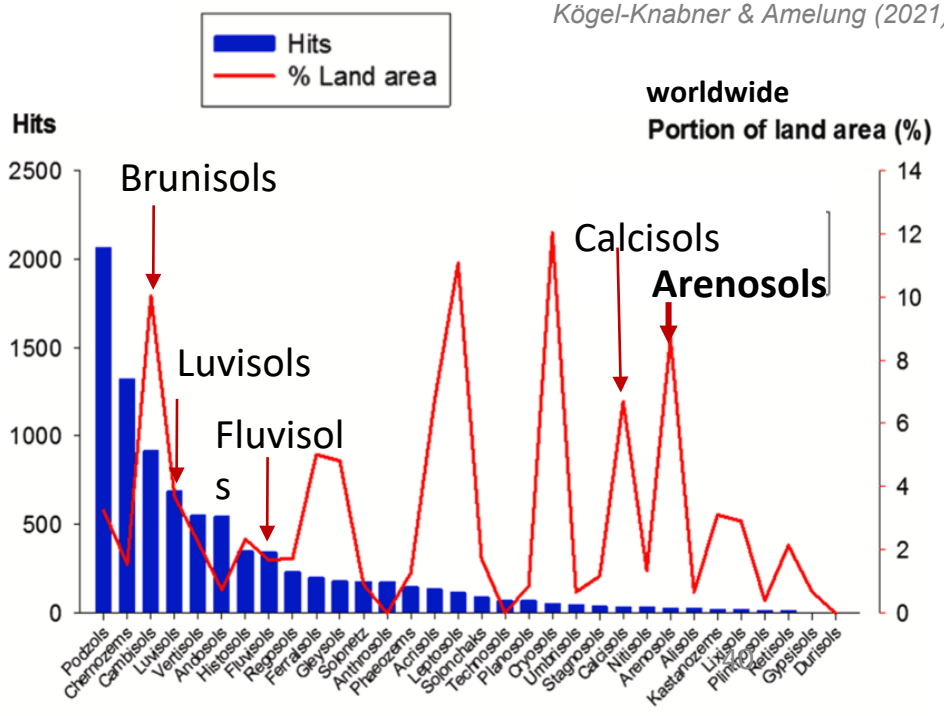
Senegal : Arenosol Bambey
- Cenozoic sandstone parent rock
- Mineralogy dominated by quartz
- 12% clays +silts / 88 % sands
- TOC = 4 gC/kg

(proportion in France)

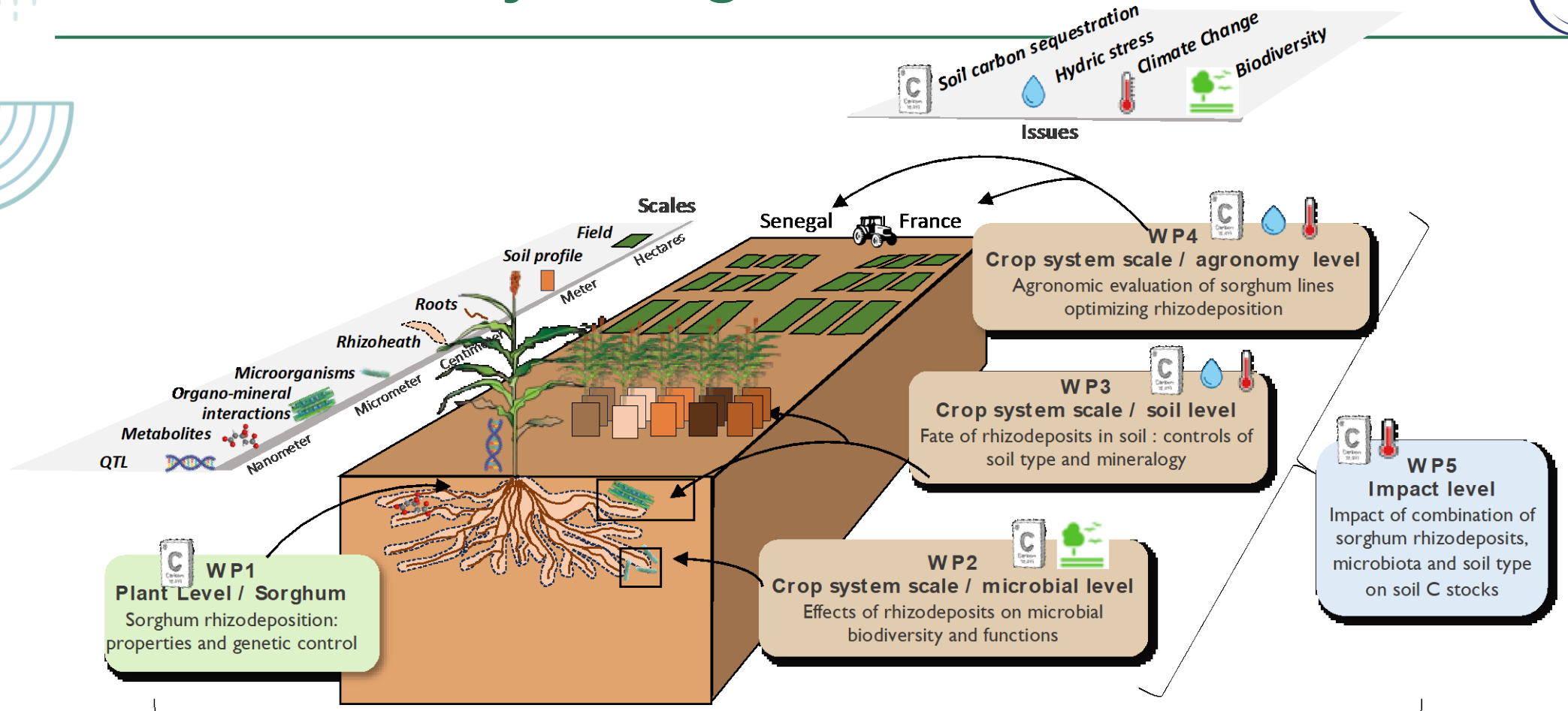
- **Brunisols** (19,4 %)
- **Calcosols** (10.8 %)
- **Luvisols** (7%)
- **Fluvisols** (6,3%)



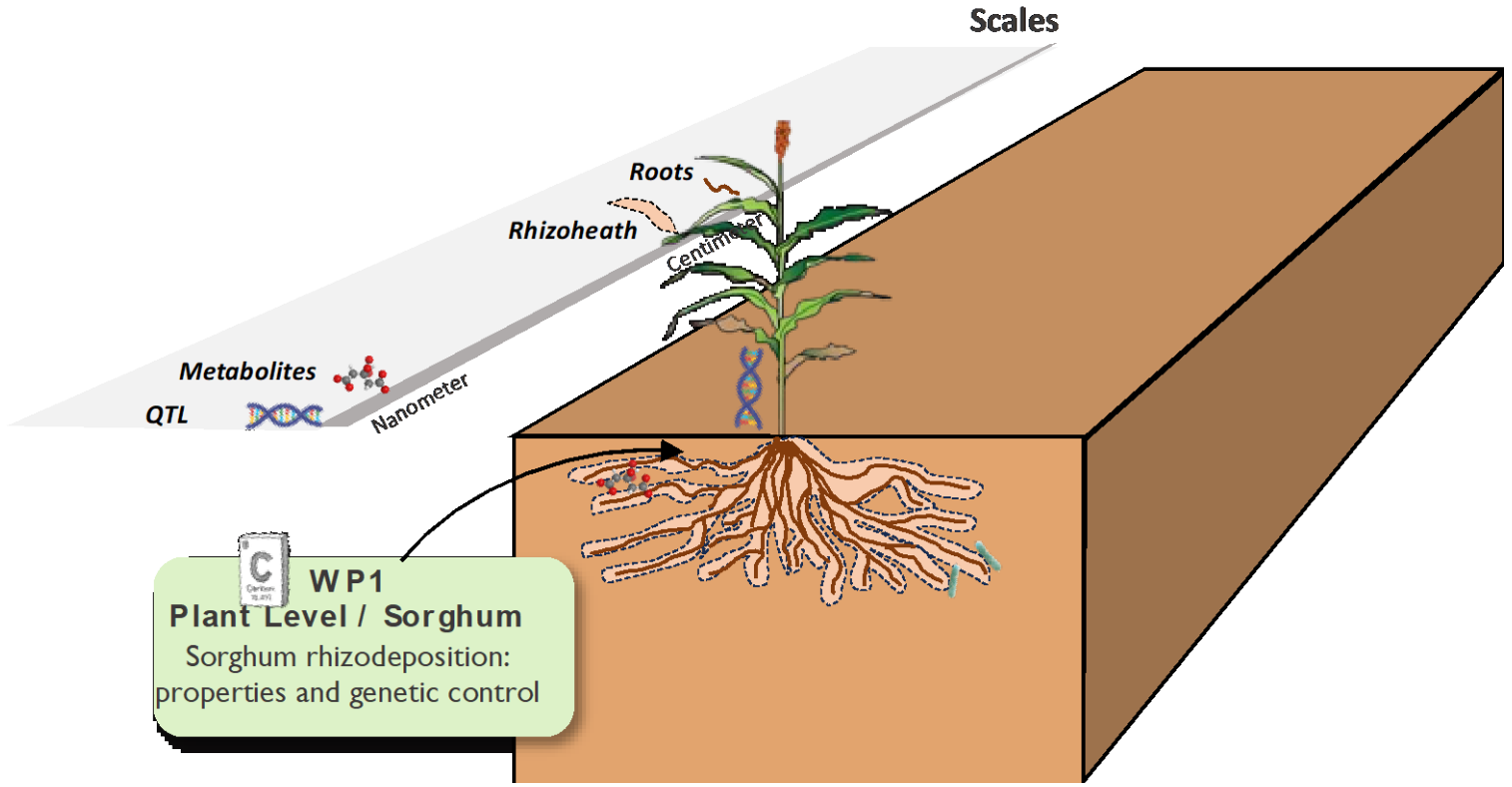
Kögel-Knabner & Amelung (2021)



Project organization



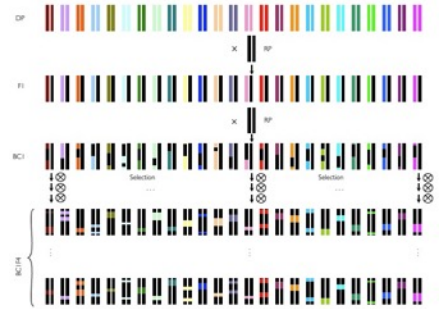
WP1 Plant Level: biomass rhizodeposition and soil rhizosheath formation in contact with the roots (L. Laplaze)



Task 1: Genetic diversity for rhizosheath formation in sorghum

(L. Laplaze, L. Cournac)

- Characterization of parental lines (12-30) and improved varieties
- Phenotyping of 300 families from 3 crosses (most relevant parental lines) Genomic region identification (QTL)



→ Contrasted soil aggregation lines for other tasks and WP

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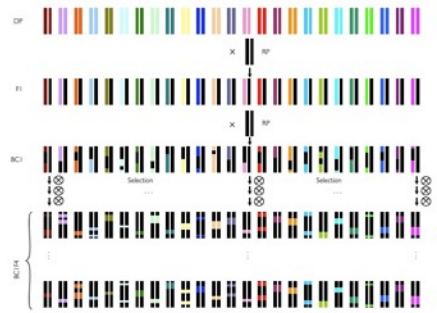

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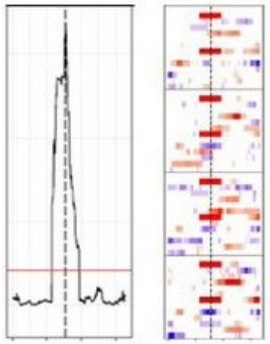
Task 2: Genetics of root soil aggregation
(D. Pot, L. Laplaze)

- Fine mapping within the most important genomic region
- Population development
- Phenotyping
- OMICS integration
- Selection of « elite » contasting lines



→ Candidate genes
→ Breeding tools

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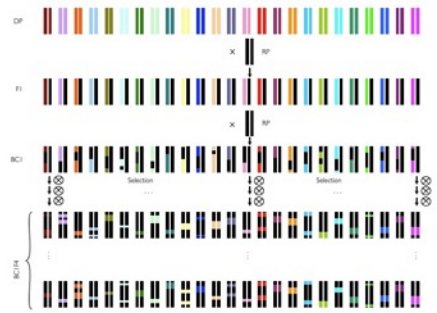

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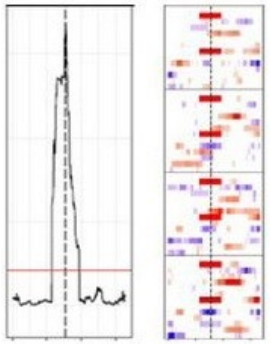

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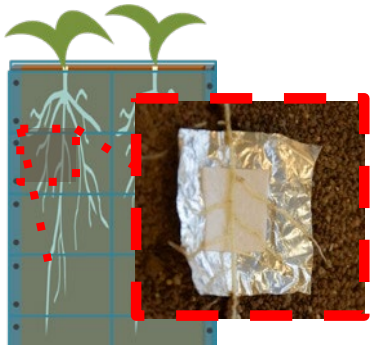
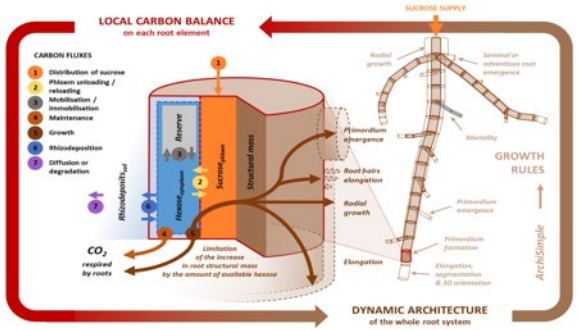
Task 3: Quantifying total rhizodeposition on selected genotypes
(F. Rees)

- Root architecture monitored by time-lapse image analysis
- Root metabolite contents destructively analyzed
- RhizoDep plant model





→ whole-plant rhizodeposition fluxes

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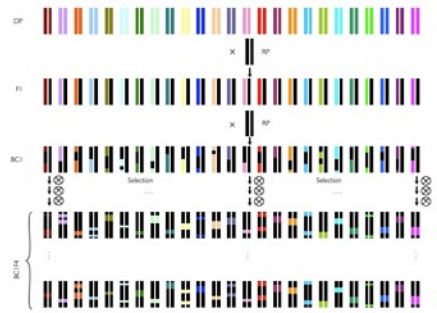

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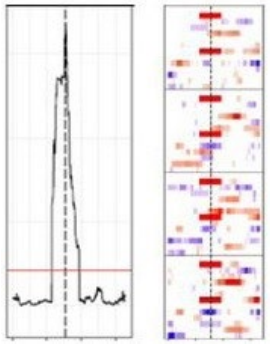

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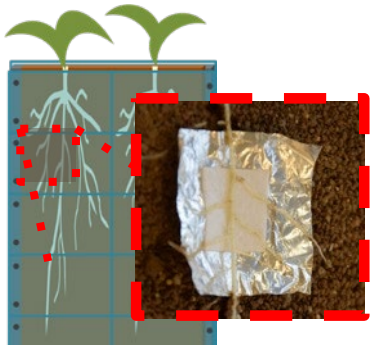
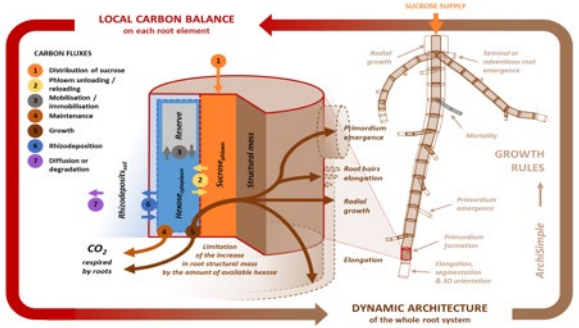




→ whole-plant rhizodeposition fluxes

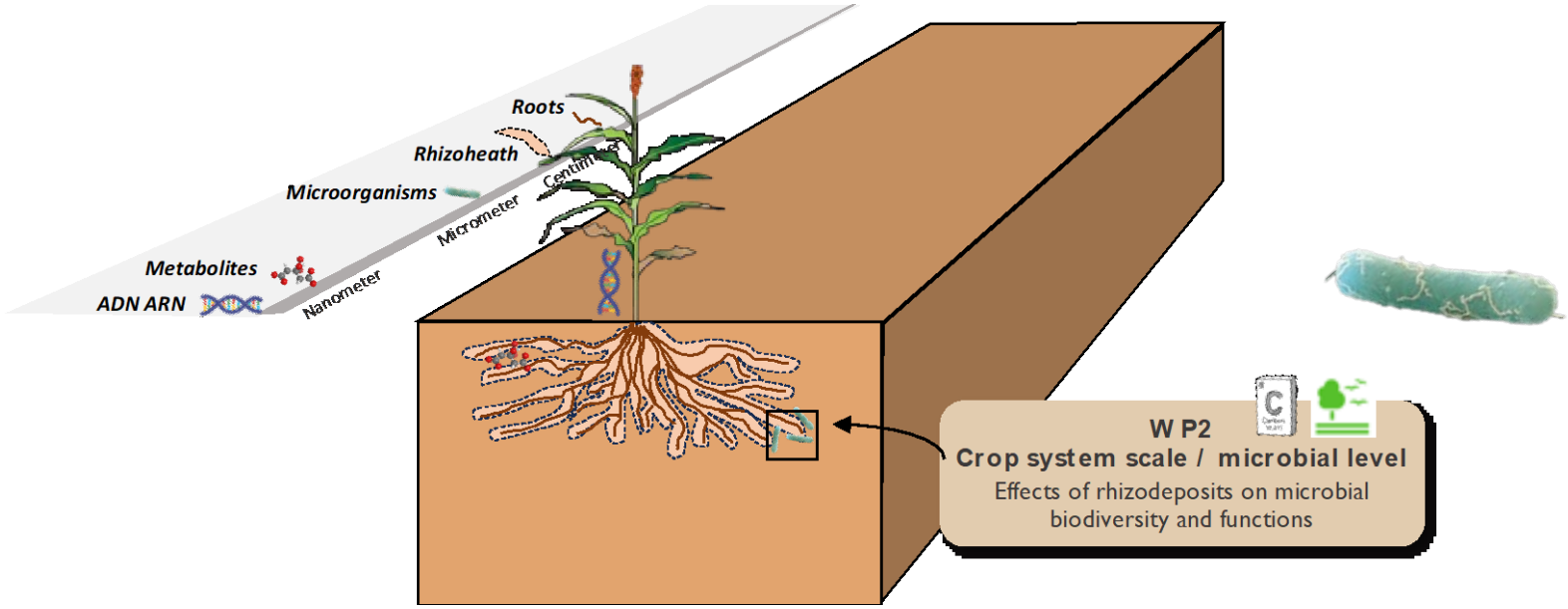
Task 4: Characterization of root exudate composition
(W. Achouak)

- Untargeted metabolic profiling of the bulk soil, the rhizosheath and the whole plant
- MetaboAnalyst server

FTICR-MS

→ Rhizodesposited metabolites / microbiote control

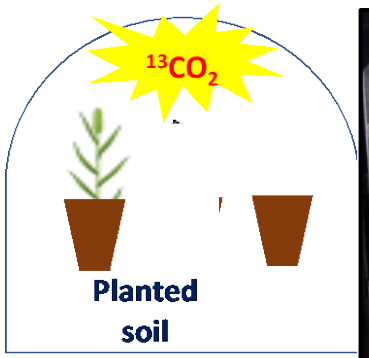
WP2: Crop system level: biomass rhizodeposition controls the soil microbial diversity (W. Achouak)

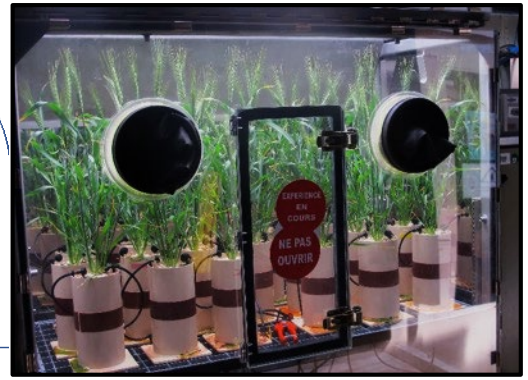


Task 1: Characterization of the biodiversity of microbial communities involved in recent and ancient carbon cycling

(W. Achouak)

- stable isotope probing (SIP) to trace structure and functions of active microbial communities
- PCR, using primers targeting bacterial 16S rRNA (V3-V4) and the ITS2 internal transcribed spacer region for fungi
- microbiome analyses using open-source QIIME2 software.





→ taxonomic identification of bacteria and fungi (RAS and SOM assimilation) : control by rhizodeposition

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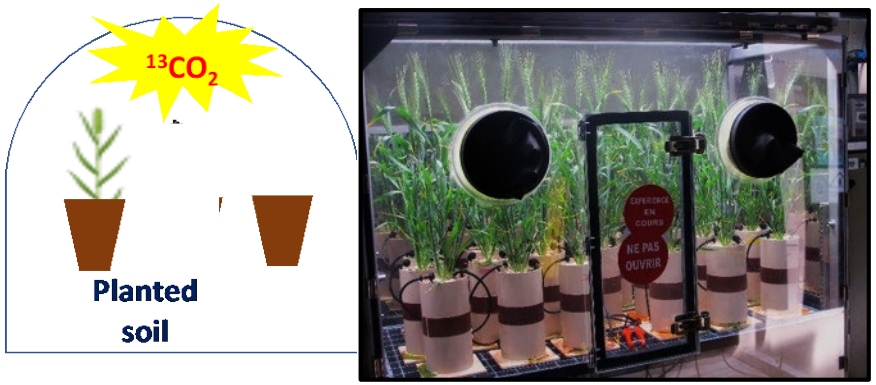

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Task 2: Gene expression patterns of root exudate assimilators and soil organic matter degraders

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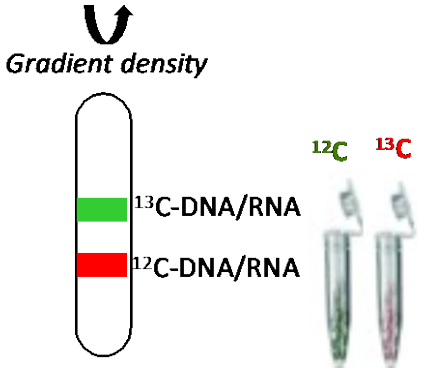
- RNA-SIP with metatranscriptomic to analysis regulation of bacterial gene expression
- DNA sequenced using the Nextera dual-indexed DNA sample preparation kit on the Illumina RNAseq (HiSeq 2500) system.
- TRINITY software with default parameters or the MGniftool,



→ structure and functions of microbial populations involved in exudate consumption

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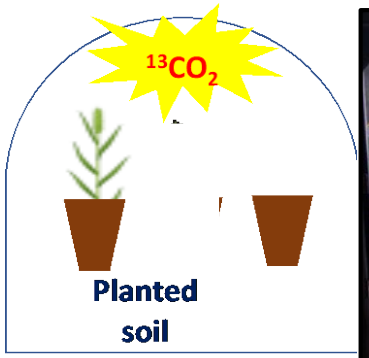

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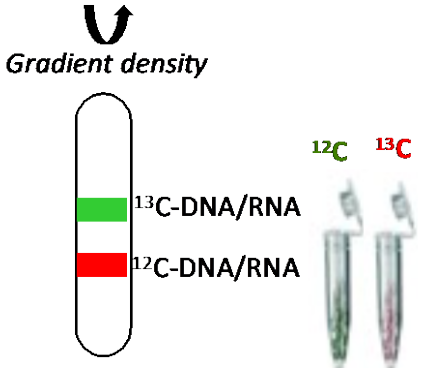
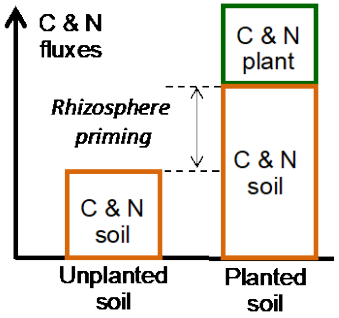


→ structure and functions of microbial populations involved in exudate consumption

Task 3: Plant root effects on soil C & N cycling via the acceleration of soil organic matter decomposition

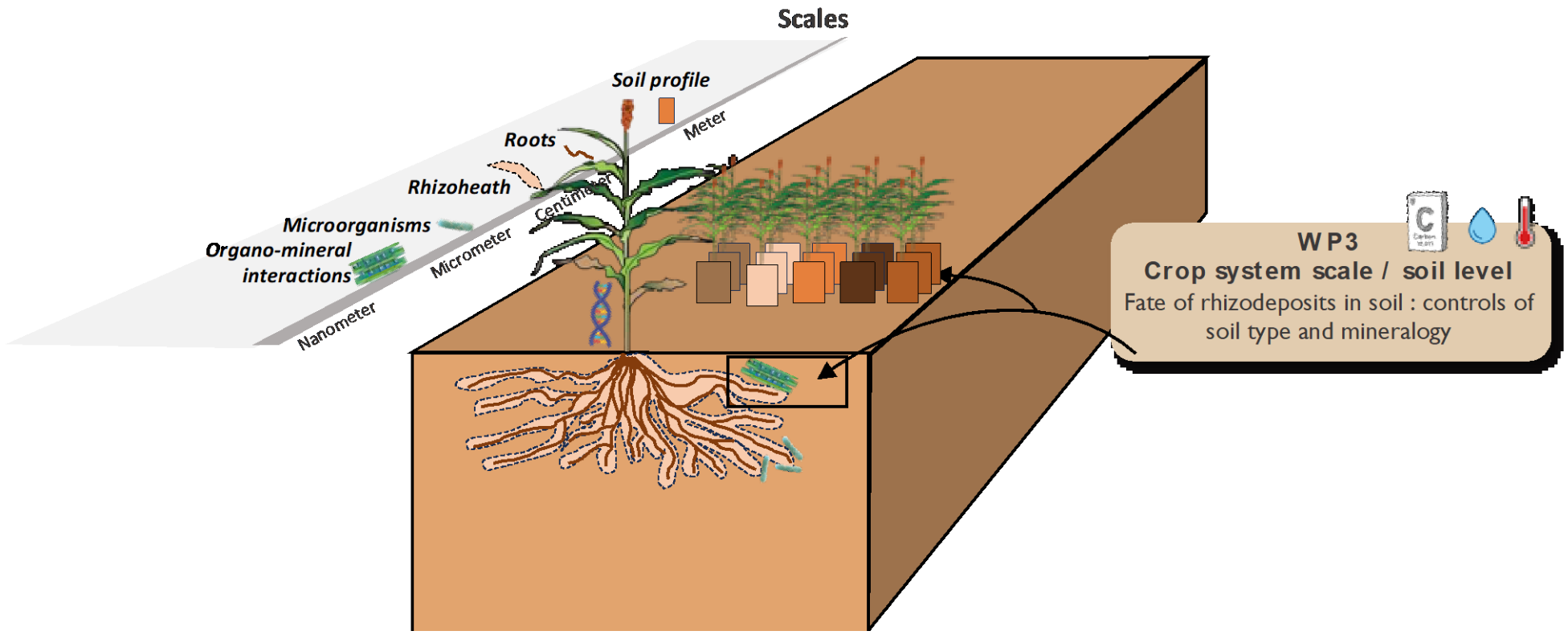
(L. Henneron)

- Priming effect measured by CO2 concentration and $\delta^{13}C$ (GC-IRMS)
- gross organic N mineralization and nitrification fluxes using ^{15}N isotope pool dilution (IPD) methods



→ C and N cycling as a function of rhizodeposition

WP 3 Crop system level: fate of biomass rhizodeposition and its control by soil type and soil mineralogy (I. Basile-Doelsch)



Task 1: Influence of the soil type on soil rhizosheath formation in selected sorghum lines with contrasted rhizodeposition characteristics

(L. Cournac, L. Laplaze)

- sorghum+/- lines cultivated in greenhouse in five different soils
- root-adhering soil, plant aerial and root biomass, root architecture (winrhizo), and C contents in root-adhering and bulk soil

Brunisols
Calcosols
Luvisols
Fluvisols
Arenosol

→ effects of the soil type on the C sequestration potential

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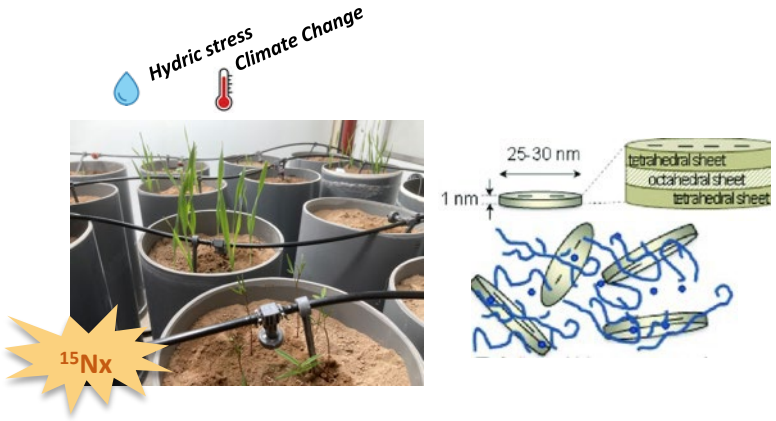
→ effects of the soil type on the C sequestration potential

Task 2: Adding clays to improve C sequestration

(S. Abiven, I. Basile-Doelsch)

- addition of highly reactive mineral phases to promote organo-mineral interactions
- quantification of C stabilization (balance between inputs and priming) using ^{13}C labelling in climatic chambers
- under 2 contrasted climates
- Stability analysed by RockEval (AMG initialization)



→ mineral control on C sequestration under climatic constraints

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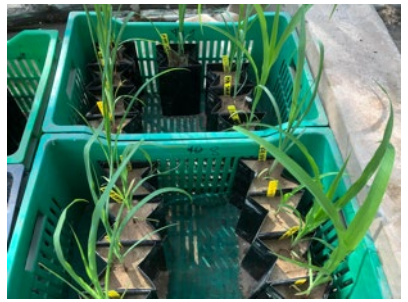
Fluvisols
Arenosol

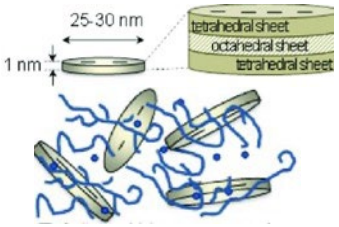


→ effects of the soil type on the C sequestration potential

Task 2: Adding clays to improve C sequestration

(S. Abiven, I. Basile-Doelsch)

- addition of highly reactive mineral phases to promote organo-mineral interactions
- quantification of C stabilization (balance between inputs and priming) using ^{13}C labelling in climatic chambers
- under 2 contrasted climates
- Stability analysed by RockEval (AMG initialization)

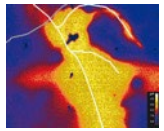


→ mineral control on C sequestration under climatic constraints

Task 3: Monitoring rhizodeposit transfer in soil using imaging methods

(I. Basile-Doelsch)

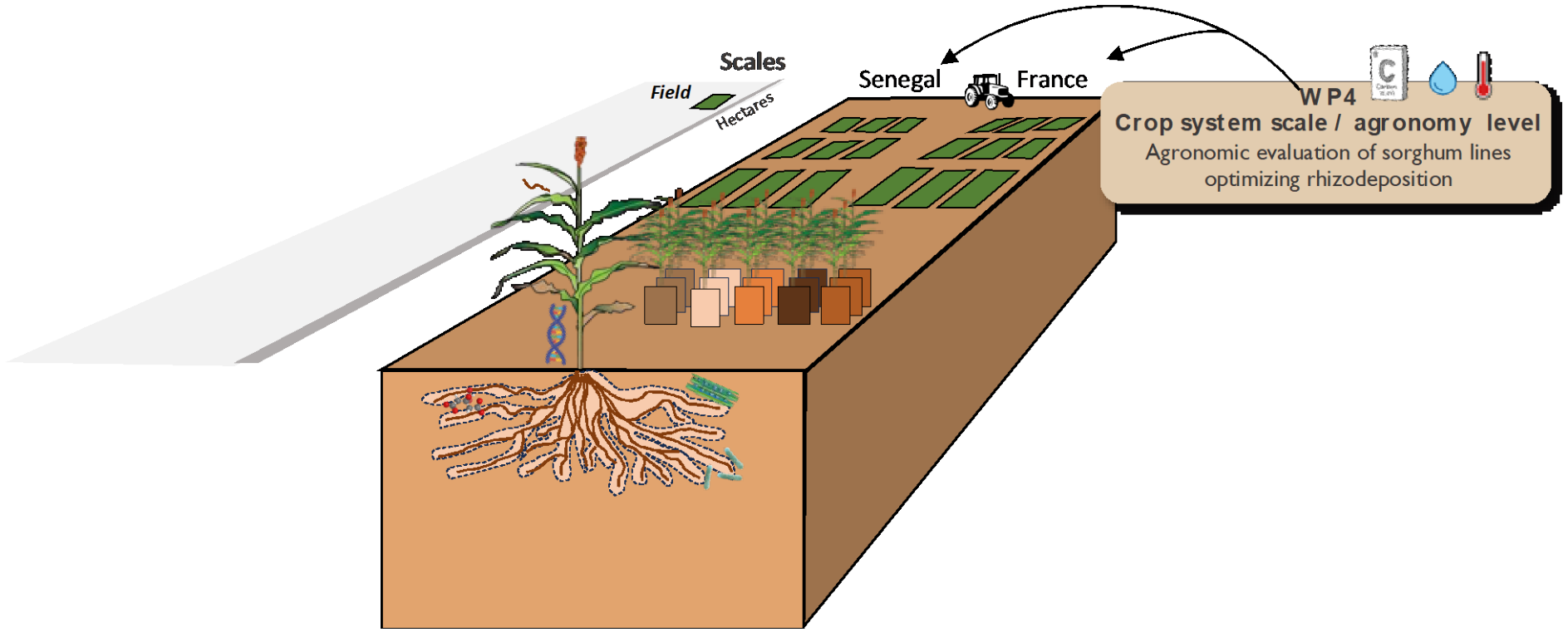
- cultivation in rhizoboxes
- monitor rhizospheric physicochemical gradients (pH, Eh) (optode)
- 2D chemical mapping (C, N, Si, Al, Fe, P, Mn, K) by new generation microXRF



Attomap microXRF

→ location of rhizodeposits in the rhizosphere / correlation with physicochemistry

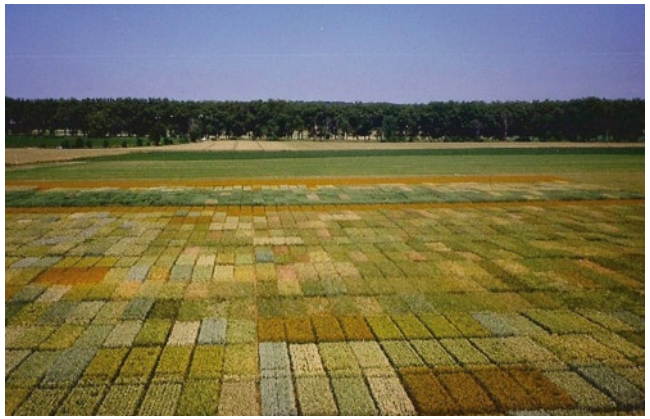
WP4 Crop system level: biomass rhizodeposition and agronomic performances (L. Cournac, L. Laplaze)



Task 1: Sorghum field trials at experimental stations in West Africa and France

(L. Cournac, L. Laplaze)

- field trials in microplots in contrasted climates : in Senegal (Bambey research station) and in France (INRAe GCA Auzeville)
- 10 improved varieties sorghum BCNAM families differing in terms of major QTL controlling root-adhering soil aggregation
- In Senegal 2 conditions : hot dry season in optimal conditions (recommended fertilization and irrigation) and in low input conditions (low irrigation, low P and combined stress)



→ impact of this increased rhizodeposition on plant performances under stress conditions in different climatic environments.

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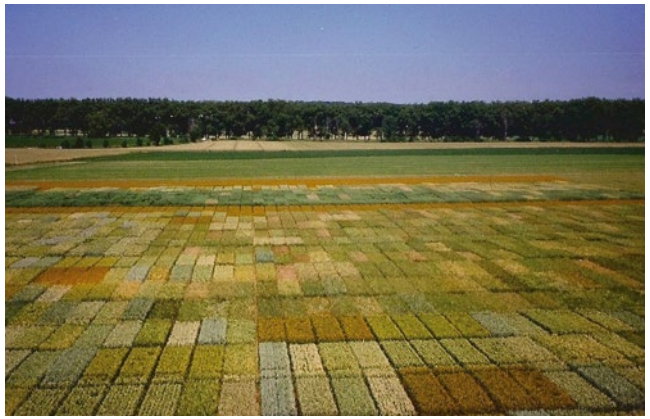

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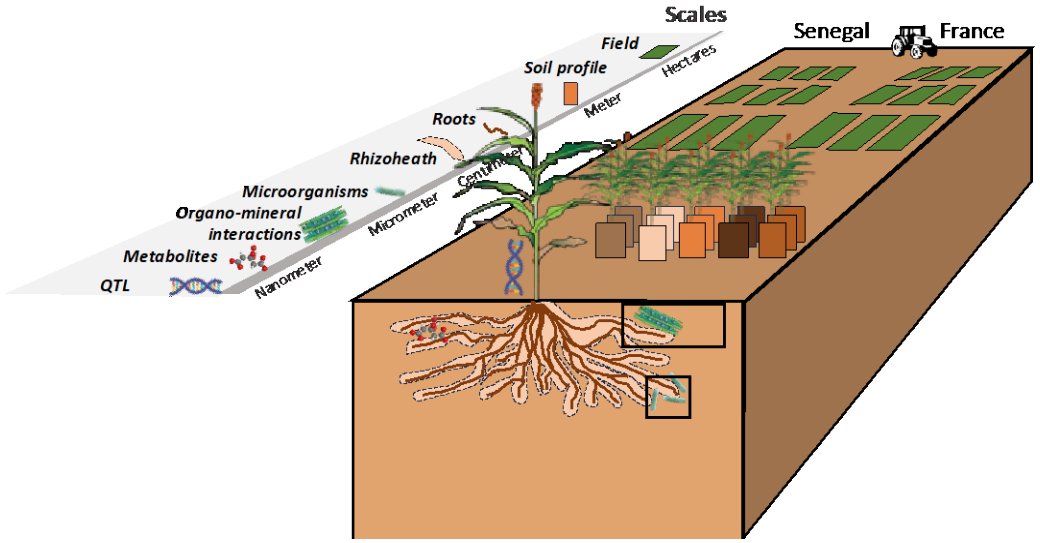
Task 2: Agronomic evaluation of contrasted sorghum lines in a farming environment (Niakhar)

(L. Cournac, L. Laplaze)

- trials in farmers' fields in Niakhar (Senegal)
- 3 years
- during the rainy season
- with the same BCNAM lines contrasting in terms of root soil aggregation

→ performance in farmers' cultivation conditions

WP5 Impact level: biomass rhizodeposition and carbon sequestration in soils (D. Derrien)



W P5
Impact level
Impact of combination of sorghum rhizodeposits, microbiota and soil type on soil C stocks

Task 1: Rhizodeposited biomass: quantification of short-term soil C sequestration

(C. Hatté, L. Henneron, S. Abiven)

- field trials in microplots at ECOTRON Ile de France
- continuous ^{13}C plant labeling based on C3-C4 plant species substitutions with ^{14}C dating and C content assessment (Ndour et al. 2022)
- monitor the amount of plant-derived rhizodesposited C added to the soil, amount of primed native C, age of primed native C, and impact of the overall carbon dynamics (storage sustainability)

ECOTRON Ile-de-France

Micadas ^{14}C measurements

→ significance and sustainability of the additional C sequestration achieved by varietal selection and/or by the addition of mineral phases

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Micadas ^{14}C measurements

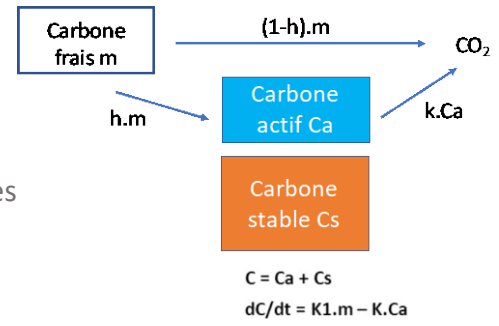
→ significance and sustainability of the additional C sequestration achieved by varietal selection and/or by the addition of mineral phases

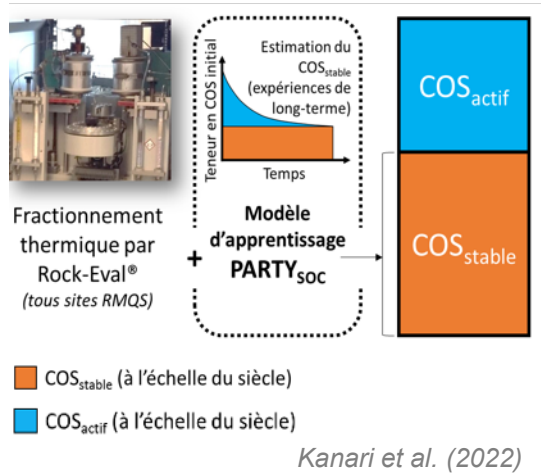
Task 2: Rhizodeposited biomass: predicting additional C sequestration in soils by 2050

(D. Derrien - C. Hatté)

- use the data and knowledge acquired in the other project WPs to test a soil C dynamics model (AMG model)
- predict soil C storage induced by sorghum cultivation over several decades (by 2050) and as a function of different IPCC climatic trajectories

AMG model : soil C dynamics





Kanari et al. (2022)

→ C sequestration : higher than the highest of business-as-usual agroecological practices (~0.5 tC/ha) ?

North / South experiments

Lab experiments WP1, WP2, WP3

ECODIV - *Rouen*

ECOSYS - *Saclay*

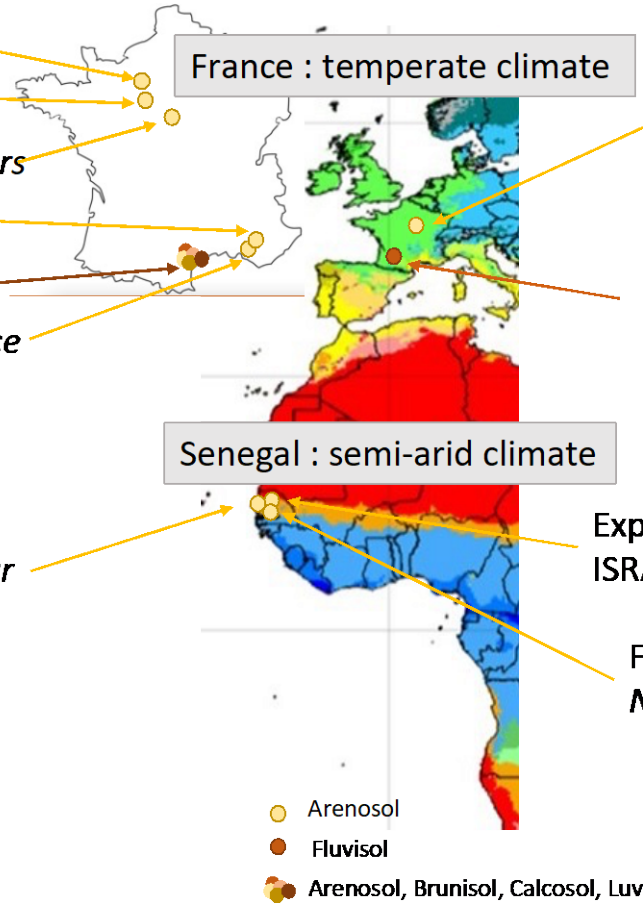
CEREEP ECOTRON - *Nemours*

BIAM - *Aix-en-Provence*

ECO&SOL - *Montpellier*

CEREGE - *Aix-en-Provence*

ISRA- *Dakar*



Köppen climate classification

Field experiments WP4, WP5

Experimental site :
CEREEP ECOTRON,
Nemours

Experimental site :
INRAE UE APC,
Auzeville

Experimental site :
ISRA- *Bambey*

Farmers
Niakhar

Sorghum BCNAM trial established in 2014

Organization of the partnership

PROGRAMME DE RECHERCHE FRANCE 2030 CARBONE ET ÉCOSYSTÈMES CONTINENTAUX

WP1 WP2 WP0

BIAM *(W. Achouak WP2)*

- *rhizosheath phenotyping*
- *rhizosphere microbiota*
- *plant microbe interaction*
- *phenotyping labeling with stable isotopes*

WP1 WP4 WP0

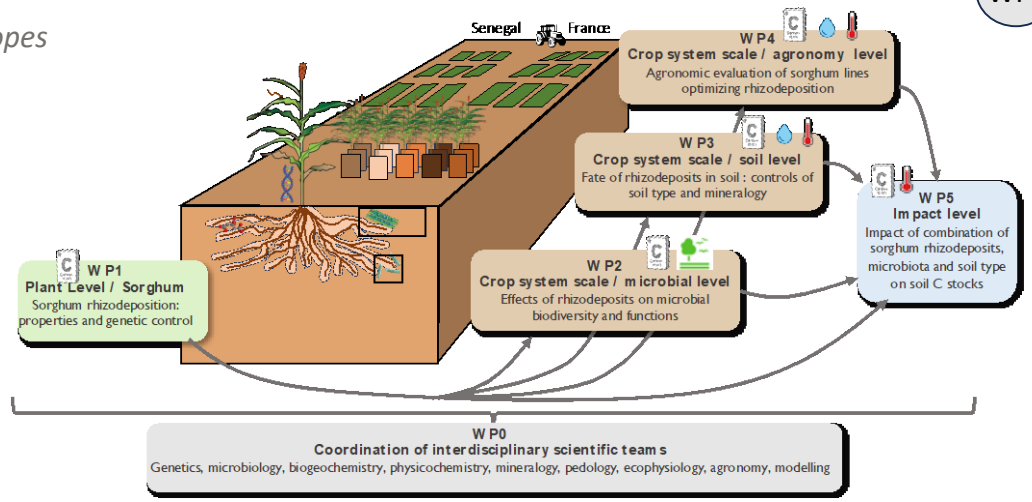
DIADE + ISRA & CERAAS *(L. Laplaze WP1, WP4)*

- *ecophysiology*
- *field phenotyping*
- *root development interaction with soil microorganisms*

WP1 WP3 WP4 WP0

ECO&SOL + INRAE APC *(L. Cournac WP4)*

- *Rhizosheath phenotyping*
- *composition and function of rhizosphere microbiome*
- *field trials in Senegal*
- *development of low-tech indicators*



WP3 WP0

CEREGE *(I. Basile-Doelsch WP3)*

- *C dynamics in soils*
- *mineralogy*
- *physicochemistry*
- *micro and nano-scales*

WP1 WP4

AGAP *(D. Pot)*

- *Population genomics of sorghum*
- *sorghum phenotyping*
- *quantitative genetics*
- *Genetics and ecophysiological modelling*

WP1

ECOSYS *(F. Rees)*

- *measurements of rhizodeposition*
- *root architecture*
- *mechanistic plant-soil modelling*

WP3

ENS-PARIS + ECOTRON *(S. Abiven)*

- *dynamics of organic matter*
- *isotope labelling*
- *radiocarbon*

WP5 WP0

BEF *(D. Derrien WP5)*

- *soil C dynamics modeling*
- *priming effect mechanisms*
- *C turnover*
- *prospective approaches for ecosystem services*

WP2 WP5

ECODIV (L. Henneron)

- *field experiments*
- *combinations of $^{13}C/^{14}C$*
- *priming effect mechanisms*
- *isotopic pool dilution (IPD)*
- *soil N cycling processes*

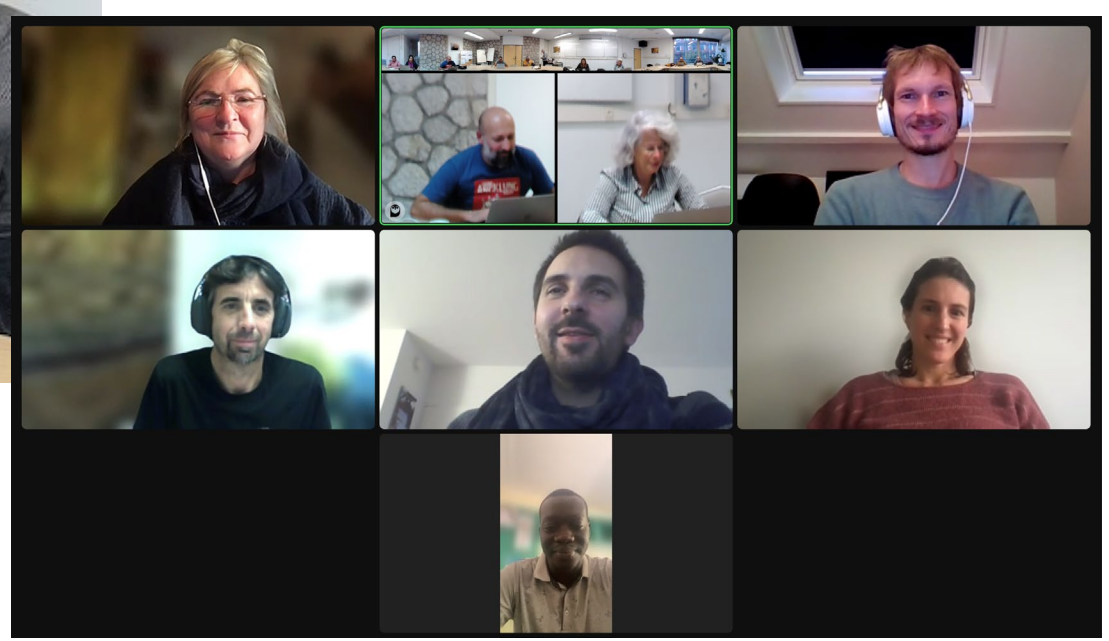
WP5

LSCE *(C. Hatté)*

- *isotopic geochemistry ^{13}C ^{14}C*
- *method of cross-calculation of isotopic ^{13}C and ^{14}C mass*
- *quantify C stocks and priming effect*

Kick-Off meeting

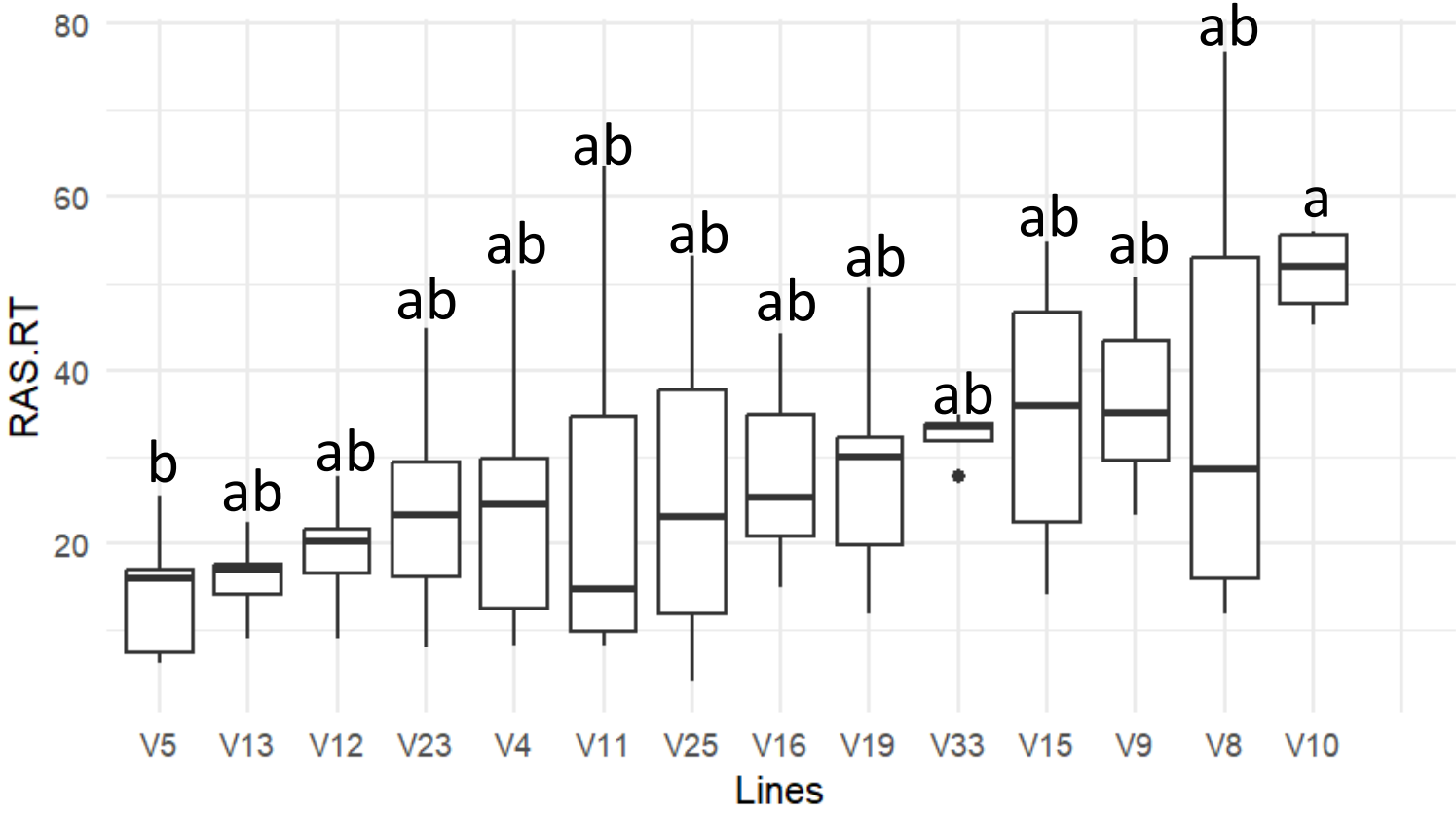
15 /12/2024 CEREGE Aix-en-Provence



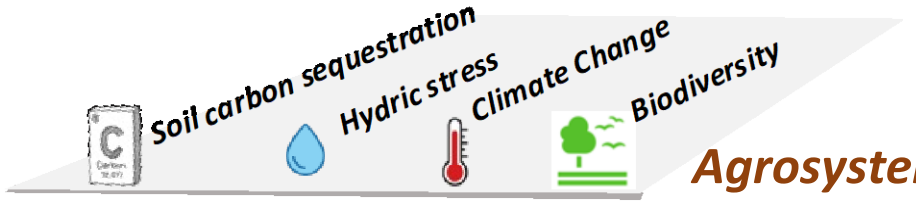
First « Seq-C » result

Ratio RAS/RT de 14 lignées de sorgho (BCNAM).

Chaque boxplot représente la moyenne de 6 répétitions. Les lettres différentes indiquent des différences significatives entre les lignées de sorgho selon le test de Kruskal-Wallis suivi du test de comparaison par paire de Dunn ($p < 0,001$).



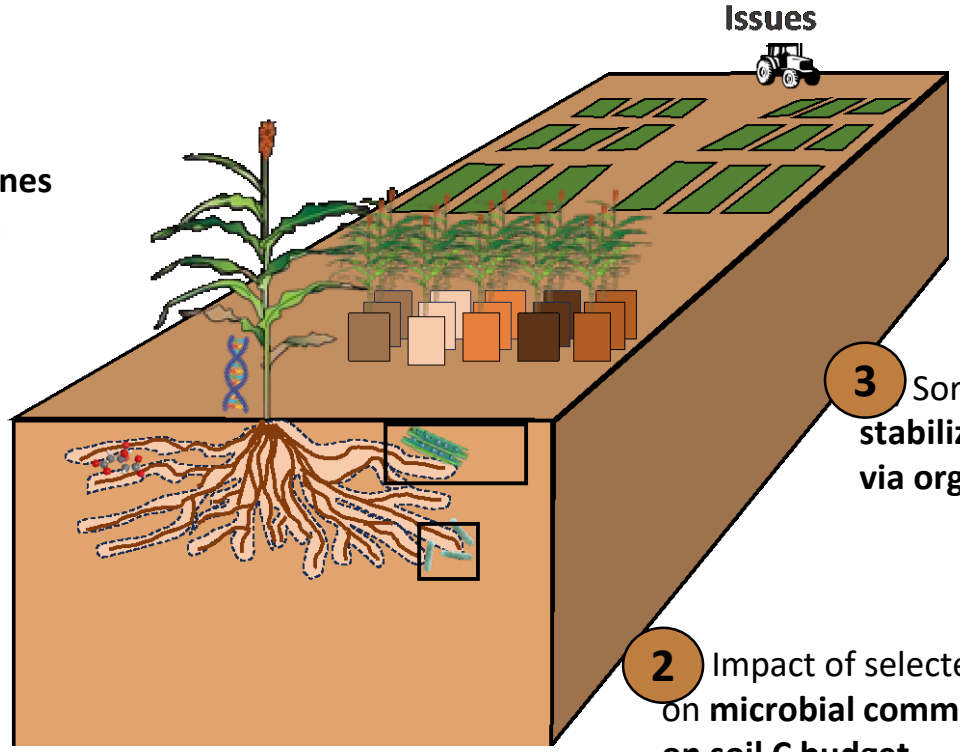
RhizoSeqC : main outcomes



Plant level

1 **Sorghum donor lines** and **genomic regions (QTL)** promoting rhizodeposited C

ANR SorDrought



Agrosystem level

4 **relevant agronomic yield and water demand in both North and South** environments

FairCarboN PC4 : CROSYEN

3 Sorghum **rhizodeposits' stabilization** in various soil types **via organomineral associations**

2 Impact of selected sorghum lines on **microbial communities and control on soil C budget**

Impact level

5 prediction of **C sequestration crops up to 2050** compared to agroecological practices

FairCarboN PC1 : ALAMOD

6 A new, structured **international multidisciplinary scientific community**

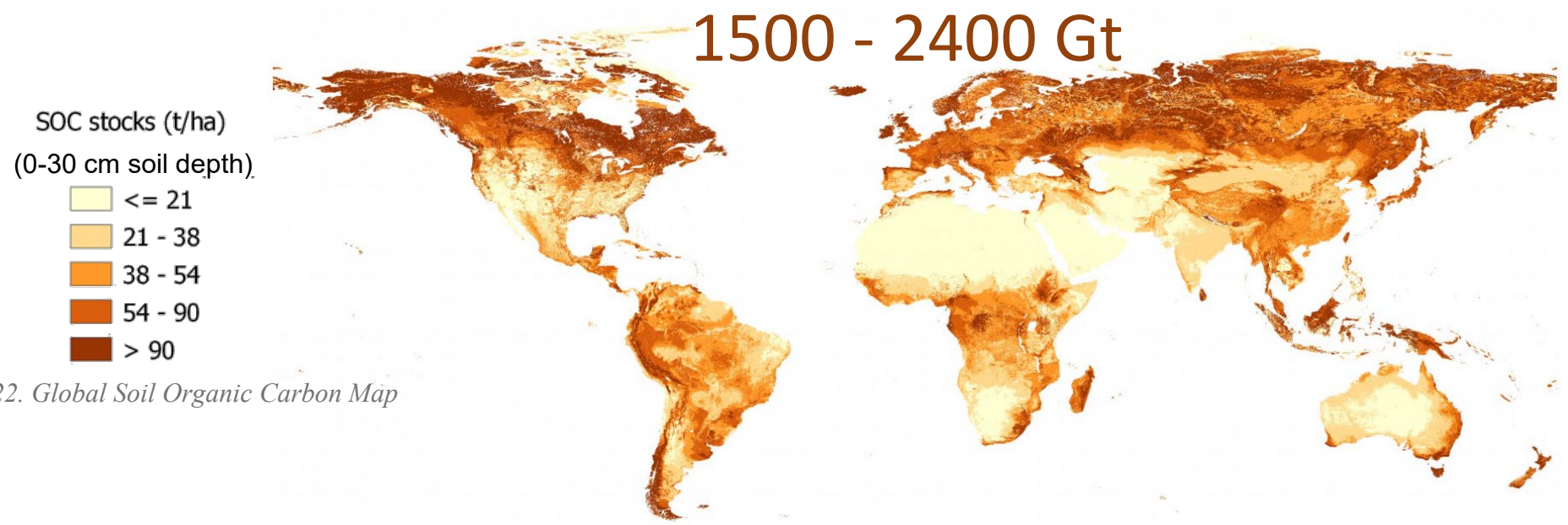
FairCarboN StakeHolders : Plant-Alliance, ADEME, IAVAO

Soil organic C stock

Atmosphere
860 Gt

Soils
1500 - 2400 Gt

Vegetation
450-650 Gt



FAO. 2022. Global Soil Organic Carbon Map

Global C debt due to agriculture for the top 2 m of soil

↘ 116-135 Gt

Sandermann et al, 2017
Lal et al., 2018